

REVIEW

**Bioelectric Control of Effector Responses in The Marine
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INTRODUCTION

All animals exhibit effector activity in response to stimuli from their external and internal environments. In multicellular animals, stimulus energy is converted into a receptor potential at the receptor membrane of a sensory cell. The receptor potential produces a train of action potentials, the sensory information, across the membrane of the sensory nerve axon, adjacent to the receptor membrane. The sensory information travels along the axon to the central nervous system, where it is integrated by means of chemo-electrical interaction between many neurons. Integration of the sensory information results in the generation of action potential in the central nervous system. These potentials are sent down motor nerve axons to effector organs. Thus the effector responds appropriately to the sensory input. Membrane electrical events, therefore, play a major role in regulating effector activities in multicellular organisms. This is also true for unicellular organisms. Unicellular organisms, however, must perform all these functions within the confines of a single cell. Nation [1] reviewed how this is achieved in some protozoans. During the course of their evolution protozoans have come to possess multiple functions in their single membrane. For example, the *Paramecium* membrane produces receptor potentials in response to external stimuli, integrates the

potentials due to the isopotential nature of its protoplasm, and generates action potentials according to the integrated level of the membrane potential. The action potentials control the motility of the cilia, and thereby the swimming behavior of the *Paramecium*. It should be noted that these different membrane functions reside in different membrane areas respectively. For example, in a forward swimming cell, a depolarizing mechanoreceptor potential is generated at the membrane of anterior end in response to a collision with a mechanical obstacle. A Ca^{2+} action potential is generated exclusively at the membrane covering the cilia when it is depolarized electrotonically by this receptor potential. The action potential brings about a transient increase in Ca^{2+} concentration within the cilia to cause a transient ciliary reversal, which results in a transient backward swimming of the cell to avoid the mechanical obstacle.

Localized differentiation of the membrane electrogenic function within a single cell is also seen in multicellular organisms. For example, the axon hillock is a generation site of action potentials in a motor neuron [2], the distal end of some sensory nerve axons are generation site of receptor potentials etc. Therefore, studies on the localized differentiation of the membrane function and its biological significance in unicellular organisms can contribute greatly to our understanding of general principles of functional differentiation within single cell.

A large marine dinoflagellate *Noctiluca* exhibits two distinct kinds of effector activity, emission of light and motile response of its tentacle. In 1957

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Hisada* [3] of Tokyo University first recorded spontaneous membrane potential perturbations through a microelectrode inserted into *Noctiluca*. In 1960 Chang** [4] of the National Institute of Health, U.S.A. examined the electrophysiological properties of a non-luminescent form of *Noctiluca*. In his admirable series of papers appearing in Science [5-7], Eckert*** of Syracuse University presented some beautiful data on the bioelectric control of the bioluminescent flash in *Noctiluca*. His papers also attracted considerable attention because of his employment of sophisticated (at least at that time) electrophysiological and photosensing equipment. Sibaoka**** of Tohoku University had joined Eckert in 1966 to perform more detailed examinations of the membrane electrogenesis and its relation to the effector activity in *Noctiluca*. After his coming back to Japan, Sibaoka continued his studies on *Noctiluca* with his student Nawata. They proposed a H^+ -mediated coupling mechanism between the membrane electrogenesis and the luminescent flash [8]. They also examined the feeding behavior of *Noctiluca* and its electrophysiological correlates [9-11].

More recently Sibaoka's student Oami joined Naitoh at the University of Tsukuba to perform detailed examinations of the membrane electrogenesis in relation to flexion-extension of the tentacle. Oami and Naitoh have been obtaining various results, some of which are consistent with those obtained by previous workers, some not. They have proposed a new hypothesis for the bioelectric control of the tentacular movement in *Noctiluca* [12].

We will review the electrophysiological studies on *Noctiluca* with special reference to the control of its effector activity and related behavior by membrane electrogenesis. Readers can refer to a famous book by Harvey [13] for an understanding of bioluminescence in general. In 1966, Eckert [14] reviewed excitation and luminescence in *Noctiluca* based mainly on his experimental results.

Sibaoka [15] reviewed electrogenesis in *Noctiluca* in Japanese. Widder [16] reviewed bioluminescence excitation in a dinoflagellate *Pyrocystis* (see also Sweeney [17]).

MORPHOLOGY AND BEHAVIORS

Noctiluca has been classified as a protozoan belonging to the order Dinoflagellida. Recent detailed examinations of its life history revealed that *Noctiluca* produces flagellated gametes in a certain phase of its life cycle similarly to dinophycean algae [18]. Plant taxonomists, therefore, have politely invited *Noctiluca* to be a member of the plant kingdom.

Noctiluca is quasi-spherical, ranging from 300 to 600 μm in diameter (Fig. 1). The larger portion of the cell interior is a vacuole filled with sap having a specific weight a little lighter than that of sea water. The vacuole, therefore, serves as a float for the planktonic life of the cell. The vacuole is covered by a thin layer of protoplasm termed the perivacuolar cytoplasm. *Noctiluca*, therefore, has two protoplasmic membranes, one facing sea water (outer membrane) and the other facing the vacuolar sap (inner membrane).

The most prominent feature of this small animal is its emission of light in response to stimulus, though the biological significance of this remains obscure. *Noctiluca* together with its luminescent dinoflagellate (or dinophycean) relatives is responsible for the spooky glow surrounding you when you swim in the quiescent warm sea on a summer night. The flash mechanism resides in the cytoplasm as scattered small particles.

As shown in Figure 1, *Noctiluca* has a minute motile projection, a tentacle (about 300 μm in length and $6 \times 10 \mu m$ in its elliptical cross-section; tn), which is used for gathering and eating its food (mostly small algae such as *Dunaliella*). The tentacle traps the algae with its sticky tip as it beats slowly, then sharply bends at its proximal region so that the tip comes into contact with the cytostome (indicated by an arrowhead) located in the deepest portion of the sulcus (a longitudinal groove of the cell). The algae are then ingested into the food vacuole (fv) in the perinuclear cytoplasmic mass (cm) by phagocytosis at the cytostome. The cyto-

* Hisada moved to Hokkaido University in 1956.

** Chang moved to Sogang University, Seoul.

*** Eckert moved to UCLA in 1969 and passed away in 1986.

**** Sibaoka moved to Kyoritsu Women's College in 1985.

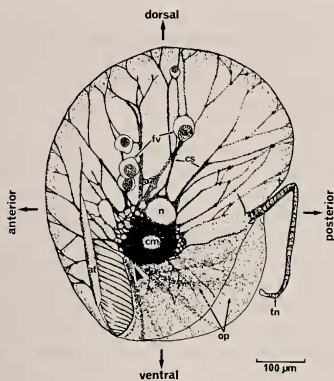


FIG. 1. Schematic drawing of lateral view of *Noctiluca*. at, apical trough; op, oral pouch (at and op form a longitudinal groove termed "sulcus"); tn, food-gathering tentacle; fv, food vacuole; cm, perinuclear cytoplasmic mass; n, nucleus; cs, cytoplasmic strand. A black arrowhead near cm indicates approximate location of the cytostome. From Nawata and Sibaoka [9].

plasmic mass contains a nucleus (n) and is connected with the perivacuolar cytoplasm by many fine cytoplasmic strands (cs). Cytoplasmic streaming towards or away from the cytostome is seen in association with its feeding behavior. The tentacle sometimes shows coiling.

EARLY ELECTROPHYSIOLOGICAL STUDIES

Summary of the first electrophysiological studies on Noctiluca by Hisada

In 1957 Hisada [3] had first recorded intracellular potentials through a microcapillary electrode inserted into *Noctiluca* (Fig. 2). Resting potential was about 50 mV negative to the external solution, when the cell was quiescent without showing tentacle movement. The electronegativity decreased with increasing external K^+ concentration as in most excitable cells, though the rate of decrease was about a half of that found in nerves and muscles (30 mV per unit decrease in logarithmic K^+ concentration).

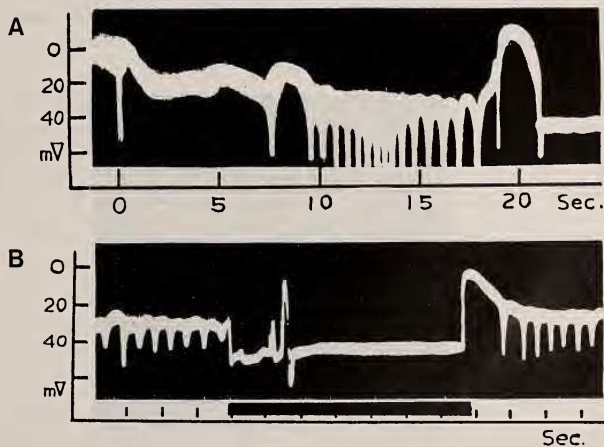


FIG. 2. A: The first records of intracellular spontaneous action potentials of *Noctiluca* correlated with tentacular activity. B: Inhibition of the spontaneous action potentials by injection of an inward current into the cell (period of the injection is indicated by a black bar below the potential record). From Hisada [3].

Trains of action potentials with varied repeating frequency (2–3 Hz at its maximum) were observed to be superimposed over a slowly fluctuating membrane potential. Strikingly, the polarity of the action potential was negative (hyperpolarizing) to the external solution, contrary to “conventional” depolarizing action potentials of nerves and muscles. Each action potential was always preceded by a depolarization of varied duration (Fig. 2A). The amplitude of the action potential was about 30 mV. More interestingly, a flexion of the tentacle was always accompanied by the hyperpolarizing action potential. Injection of an outward (depolarizing) current produced the action potential together with flexion of the tentacle upon its switching off. An inward (hyperpolarizing) current produced neither. Moreover, the repetitive action potentials were inhibited by inward current injection (Fig. 2B). Hisada could not detect the membrane potential change associated with the flash.

Summary of electrophysiological studies on Noctiluca by Chang

Chang [4] examined the electrophysiological characteristics of *Noctiluca* by employing fine glass capillary microelectrodes inserted into the flota-

vacuole and electronic equipment more sophisticated than those used by previous workers. In contrast to Hisada, no significant resting potential was observed. A hyperpolarizing action potential of all-or-none type was elicited when an inward current was injected into the vacuole and the resultant shift of the vacuolar potential (electric potential of the vacuolar sap with reference to the external solution) toward hyperpolarizing direction exceeded 100 mV (Fig. 3). The amplitude of the action potential was calculated by subtracting the Ohmic voltage shift across the membrane (i.e. current divided by electric conductance of the membrane at the peak of the action potential) from the potential shift at the peak of the action potential, and was found to be about 100 mV. The action potential was always accompanied by a conspicuous decrease in the membrane impedance within a suction hole of the experimental chamber by which the cell is arrested (about 3% of the total membrane area).

The action potential was little affected by changing ionic compositions in the external solution. This fact together with the unorthodox polarity of the action potential caused Chang to assume that the action potential might be generated in the membrane facing the vacuole.

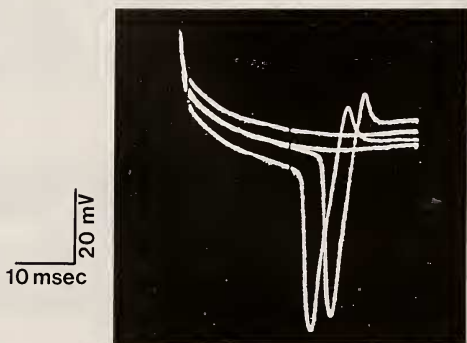


FIG. 3. Superimposed records of electric responses of *Noctiluca* to inward rectangular current pulses of four different intensities. A downward deflection of the trace corresponds to an increase in the negativity of vacuolar sap relative to the surrounding sea water. The base line of the potential trace is above the upper edge of the photograph, so it cannot be seen. From Chang [4], modified.

Membrane resistance and capacitance averaged 1.4×10^3 ohm-cm² and $1.3 \mu\text{F}/\text{cm}^2$ respectively when the cell was inactive.

Since Chang's specimens were a non-luminescent form, correlation of the action potential with the bioluminescent flash was not examined. Chang was, however, interested in flash control by membrane electrogenesis.

BIOELECTRIC CONTROL OF LIGHT EMISSION

Characteristics of the luminescent flash

Noctiluca emits light when it is stimulated mechanically, chemically, electrically or by some other means. The light flash is all-or-none and short-lived as it attains a peak amplitude 15 to 20 msec after onset, and decays to 50% in about the same time. The emission spectrum of the flash has a peak at a wavelength of 470 nm. Therefore, the flash is blue. Maximum light intensity of a single flash is about 1.5×10^{-22} watts and its total energy is about 4.1×10^{-10} joules (ca. 9.7×10^8 photons). The flash shows summation, potentiation and fatigue. For detailed kinetics of the flash see the review by Eckert [14].

Flash-triggering action potential

In a luminescent form of *Noctiluca*, Eckert [5] obtained a hyperpolarizing action potential similar to that obtained by Chang [4]. He employed a high-gain photomultiplier for monitoring the flash, and found that the flash was always preceded by the action potential by 2–3 msec (Fig. 4). Eckert, therefore, termed the action potential the "flash triggering action potential (FTP)".

Propagation of the FTP and the flash over the cell surface

Eckert [6] determined the conduction latency of the FTP by examining FTP-associated localized currents measured at three different membrane areas. One near the stimulation site, a second one 90 degree around the cell and a third one approximately opposite the stimulation site. As shown in Figure 5B, the latency for the peak of the membrane current was shortest with the electrode

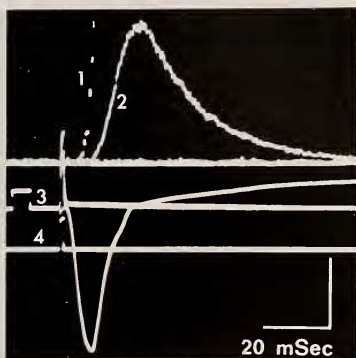


FIG. 4. The luminescent flash and the flash-triggering potential (FTP) of *Noctiluca*. Two sweeps of four traces each, one sweep with external stimulating current (monitored on trace 4) below threshold and a second sweep with suprathreshold current. The flash is converted into an electric signal by a photomultiplier and monitored on traces 1 and 2. Trace 1 is superimposed on trace 2 and displays the same signal at 20 times the gain of trace 2. Trace 3 displays the potential recorded with a microelectrode from the vacuole. Calibration pulse at beginning of this trace is 10 mV and 5 msec. Vertical calibration mark: 9×10^5 photons/msec for trace 1, 1.8×10^7 photons/msec for trace 2, 32 mV for trace 3, and 4×10^{-4} amp. for trace 4. From Eckert [5].

closest to the stimulus site and longest with the furthest one. From the latency difference between the two extreme recording sites the propagation velocity of the FTP along the circumference of the cell was estimated to be about 60 mm/sec. He also determined the light emission latency at three different cellular locations corresponding to the membrane areas where the conduction latency was measured. As shown in Figure 5A, emission latency was least when the recording location was next to the stimulus site and greatest when it was opposite the stimulus site. The propagation velocity calculated from the latency difference is almost identical with that of the FTP. These findings indicate that the flash initiated at the stimulation site spreads as FTP spreads all over the cell.

Eckert [7] precisely examined localized sources of the flash on a highly magnified image of a

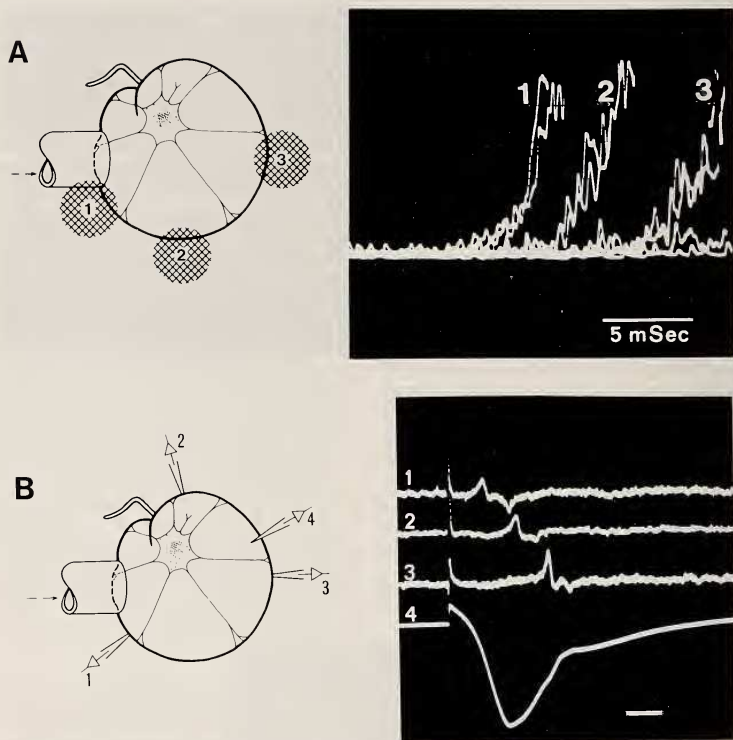


FIG. 5. Emission latency and conduction latency of the FTP in *Noctiluca*. A: Emission latencies from three restricted photometer fields at three different distances from the stimulus site. Photometer fields are indicated by shading with numbers on the left-hand diagram. 1; closest to the stimulus site, 2; about 90 degrees around the cell from the stimulus site, 3; opposite the stimulus site. The three traces with respective numbers on the right are high-gain photometer recordings of the flash from the photometer fields with corresponding numbers. B: Conduction latencies of the flash-triggering action potential. The potential is recorded through three extracellular suction electrodes placed at three different portions of the cell surface, which are shown on the left-hand diagram. Trace 4; The FTP recorded from the vacuole. The numbers beside the electrode diagrams and the traces correspond to those in A. From Eckert [6], modified.

flashing *Noctiluca* and found that fluorescent inclusions ranging from $0.5\text{--}1.5\ \mu\text{m}$ in the perivacuolar cytoplasm were responsible for the flash. He termed a flash from each microsource a "micro-flash", and a flash from a whole cell, which is a

sum of all the microflashes, a "macroflash".

Generation site of the FTP

Eckert and Sibaoka [19] successfully recorded the FTP from the perinuclear cytoplasmic mass.

The FTP was diphasic in contrast to the monophasic FTP from the vacuole. This suggests a propagation of the FTP along the inner membrane. As a matter of fact, difference between the FTP recorded from the vacuole and that from the cytoplasmic mass, (which corresponds to the FTP recorded from the cytoplasm with reference to the vacuole) showed a depolarizing polarity similar to an orthodox action potential of general excitable cells. Thus it is concluded that the FTP is an orthodox action potential generated across the inner (vacuolar) membrane of *Noctiluca*.

Ionic mechanism of the FTP and coupling between the FTP and the flash

By making use of a pH-sensitive microelectrode inserted into the vacuole, Nawata and Sibaoka [20] determined the vacuolar pH to be as low as 3.5, a value similar to that obtained by previous workers

[21-23]. They also determined concentrations of major ions in the vacuolar sap, such as Na^+ , K^+ , Ca^{2+} , Mg^{2+} , NH_4^+ and Cl^- . Concentrations of these ions were almost identical with those in sea water except for the lower Mg^{2+} concentration and the presence of NH_4^+ ions.

Nawata and Sibaoka [8] injected HCl-glycine pH buffers with different pH values ranging from 2.5 to 3.7 into the vacuole. The buffer's volume was 10-30% of the vacuolar volume. The effects of vacuolar pH on the amplitude of the FTP were then examined. The lowering the pH increased the amplitude at a rate of 58 mV/unit pH change. Ion species concentrations in the vacuolar sap other than that of H^+ showed no conspicuous effects on the amplitude. These results indicated that the FTP was dependent on H^+ ions.

Using the procedures exploited by Fogel *et al.* [24] to extract the scintillons (luminescent parti-

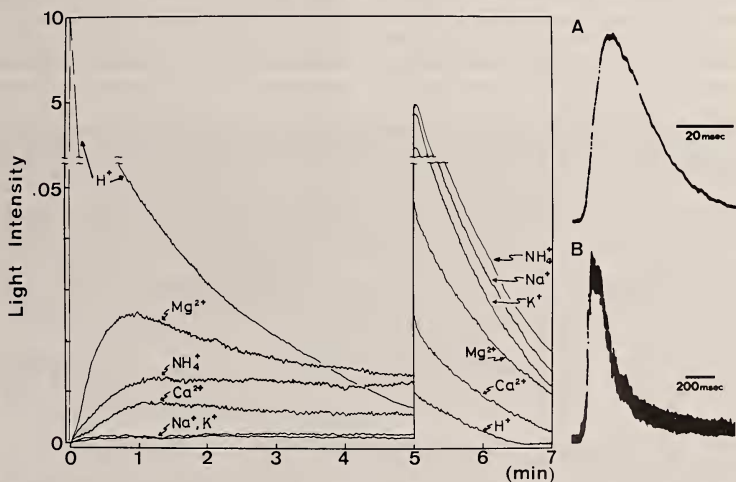


Fig. 6. Time-decay of the luminescence of the cell-free extract of *Noctiluca* after its activation. The extract was mixed with each of the various solutions with different cation species (H^+ , Mg^{2+} , NH_4^+ , Ca^{2+} , Na^+ and K^+) at time 0. The H^+ -containing solution (pH 5.1) was added 5 min after mixing. The light emission from the mixture was monitored. Ordinate: light intensity relative to the maximum intensity (regarded as 10) of the light emitted immediately after mixing the extract with the pH 5.1 solution. CRO-traces on the right of the figure are the flash from a living cell evoked by FTP (A), and that from the cell-free extract evoked by lowering pH to 5.1 (B). From Nawata and Sibaoka [8].

cles) of *Gonyaulax*, a relative of *Noctiluca*, Nawata and Sibaoka [8] prepared crude extracts of the luminescent particles of *Noctiluca*. The extract showed luminescence when the pH was lowered by mixing it with pH buffer (Fig. 6). Maximum light was obtained at pH 5.0–5.5. The peak wavelength of the emission spectrum was about 470 nm, the value identical with that of the flash in live specimens. Mixing of the extract with solutions containing Mg^{2+} , NH_4^+ , Ca^{2+} , Na^+ or K^+ produced no light emission except for a dim glow (less than 0.05% of the maximum light intensity).

These data strongly supported the hypothesis of the control of bioluminescence by membrane excitation. That is, voltage-sensitive H^+ channels in the inner membrane of *Noctiluca* are activated by a mechanoreceptor potential elicited in the inner membrane by a mechanical stimulus. H^+ in the vacuole diffuses down its electrochemical gradient through the activated channels into the cytoplasm, causing a lowering of the cytoplasmic pH. The lowered pH brings about activation of the flash mechanism in the cytoplasm, which, in turn, causes the flash. Hastings and his coworkers [25–27] had confidently predicted that *in vivo* flashing of *Gonyaulax* must be caused by H^+ movement into the scintillon in response to cellular excitation.

BIOELECTRIC CONTROL OF TENTACLE MOVEMENT

Tentacle regulating potentials

Flexion-extension of the tentacle occurs in close association with spontaneous membrane potential perturbations [3]. Eckert and Sibaoka [28] termed the potential perturbations "tentacle regulating potentials (TRPs)". The basic wave form of the TRPs consists of a slow depolarization to a level of -10 mV (pre-spike positive wave), a hyperpolarizing spike with a peak level of -85 mV (spike) and a more or less stable potential level of -45 to -60 mV (post-spike negative level). Recently Oami *et al.* [29] found that a depolarizing spike could be seen immediately before the slow depolarization, when the external Ca^{2+} concentration was lowered. The spike had been ignored by previous workers, since it is so obscure in normal sea water. Oami *et al.* [29] termed the depolarizing spike the "positive spike" and the hyperpolarizing spike the "negative spike" (Fig. 7). Recent voltage-clamp experiments [30] demonstrated that a transient small inward current corresponding to the positive spike was always evoked by a membrane depolarization even in normal sea water. The positive

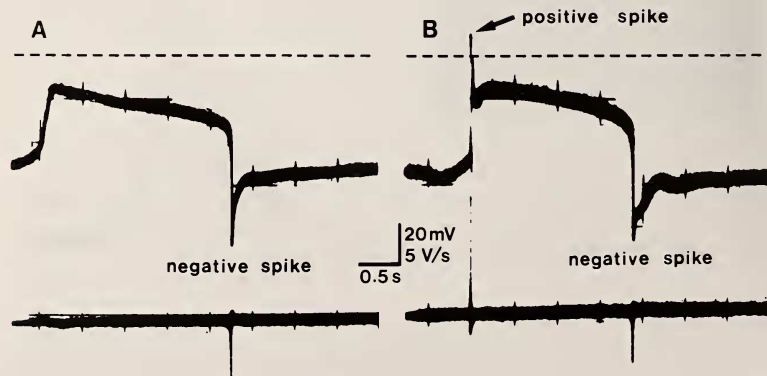


FIG. 7. The tentacle regulating potentials (TRPs) recorded through an electrode inserted into the flotation vacuole of *Noctiluca* in normal ASW (A) and in Ca-deprived ASW (B). Lower traces, first-order time derivatives of the potential shown in upper traces; dotted line, reference level for the potential. From Oami *et al.* [29].

spike, therefore, is a normal component of the TRPs.

Temporal correlation of the TRPs with the tentacle movement

Based on their simultaneous recordings of membrane potential and tentacle movement (Fig. 8), Eckert and Sibaoka [28] examined the temporal correlation of the TRPs with the tentacle movement. They found that the tentacle starts to flex within 1 sec after the beginning of the slow depolarization, and continues to flex throughout it. Extension of the tentacle takes place 1–2 sec after the negative spike. Recently we found a quick acceleration of the flexion to be associated with the negative spike [29, 31]. This finding is consistent with the early observation by Hisada [3]. Eckert and Sibaoka [28] overlooked the quick flexion. In their experiments, *Noctiluca* exhibited a rather long slow depolarization, during which the degree of the flexion reached its maximum. Therefore, enhancement of the flexion by the negative spike was obscure. Correlation of the positive spike with the tentacle movement has not yet been observed.

Generation site of the TRPs

Sibaoka and Eckert [32] pointed out that the unorthodox polarity of the TRPs could be seen as a conventional depolarizing spike with a plateau potential (such as a cardiac action potential) if the TRPs were assumed to be generated in the inner membrane. That is, the pre-spike positive wave bears similarity to a pace maker potential, while the negative spike and the post-spike negative level resemble a prolonged action potential. However, Oami *et al.* [29] found that the wave form of the TRPs recorded from the cytoplasmic compartment was identical with those recorded from the vacuole, but there was a small DC potential difference (20–30 mV) between these two potential records (Fig. 9). This indicates that the TRPs are generated in the outer membrane. Insertion of the electrode into the nucleus was a key technique for successful recording of the TRPs from the cytoplasmic compartment. Sibaoka and Eckert [32] also recorded the TRPs from the perinuclear cytoplasmic mass. The wave form of the TRPs was identical but smaller (1/2–1/4) in its

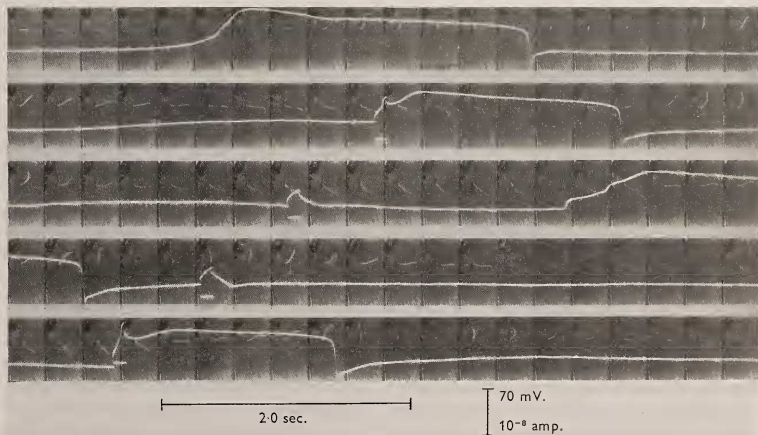


Fig. 8. Concurrent records of the tentacle regulating potentials and tentacle position in *Noctiluca*. Each frame was exposed stroboscopically at about 3/sec on continuously moving film. The potential trace was photographed from the face of a CRT. From Eckert and Sibaoka [28].

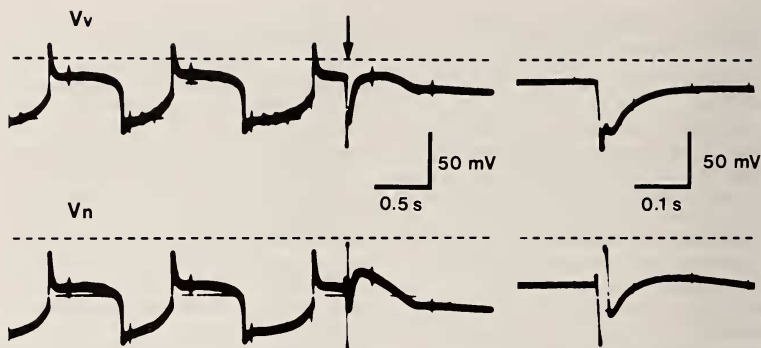


FIG. 9. Potential responses recorded simultaneously from the flotation vacuole (V_v) and from the nucleus (V_n) of a single specimen of *Noctiluca* in Ca-deprived ASW. The arrow on the V_v trace indicates the time when an inward current pulse was injected into the cell through the holding pipette to evoke a flash-triggering potential (FTP). The FTP recorded with a faster sweep (5 times as fast) is shown to the right of each trace. Dotted lines, reference levels for the potential. From Oami *et al.* [29].

amplitude than that recorded from the vacuole. We also obtained the TRPs with smaller amplitude when the electrode tip was outside the nucleus. In this state, the amplitude was unstable and became smaller with time probably due to encapsulation of the electrode tip by the streaming cytoplasm. The DC potential difference is assumed to be an algebraic sum of the potential difference across the nuclear membrane and that across the inner membrane.

Ionic mechanism for the TRPs

Oami *et al.* [29] examined effects on the TRPs of various kinds of ions in the external solution. Replacement of the standard solution with an experimental solution containing or deprived of certain kinds of ions quickly affected the TRPs. The speed of the effects of external ions on the TRPs also support the idea that the TRPs are generated across the outer membrane. The peak value of the positive spike increased with increasing external Na^+ concentration, and peak negativity of the negative spike increased with increasing external Cl^- concentration. Therefore, it can be said that the positive spike is Na^+ -dependent, and the negative spike Cl^- -dependent. Ionic mechan-

isms for the slow depolarization and for the post-spike negative level remain unsolved. As already mentioned, the peak value of the positive spike increased with lowering external Ca^{2+} concentration. This indicates that Ca^{2+} is not a current carrier but a modifier of the Na^+ -dependent positive spike.

Localized distribution of the ionic channels

Sibaoka and Eckert [32] found a conspicuous decrease in the membrane impedance near the base of the tentacle in association with the negative spike (Fig. 10). Localized outward electric current accompanied by the negative spike was observed only through the membrane near the base of the tentacle. From these results, they concluded that the negative spike is generated exclusively at the base of the tentacle. Contrary to their results, we [33] demonstrated that the localized outward current was observed anywhere on the cell surface in association with the negative spike, though the current intensity was the largest around the base of the tentacle. This indicates that the TRPs are generated anywhere on the cell surface. Sibaoka and Eckert [32] used a suction pipette thinner than ours. It is presumable that a larger negative

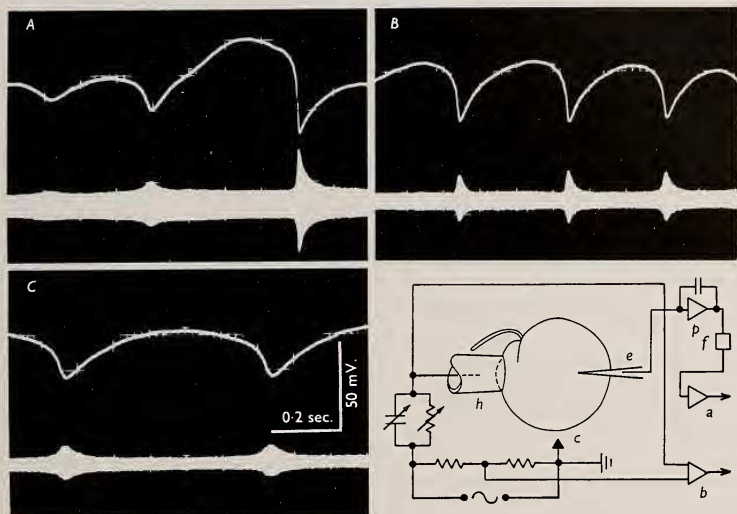


Fig. 10. Impedance changes associated with the negative spikes of the TRPs with three different patterns recorded in one and the same specimen. Upper trace: vacuolar potential. Lower trace: bridge balance. Increments in the trace width are proportional to the impedance change. Schematic of experimental set-up is shown in the bottom right: h, holding pipette having an internal diameter of about $100 \mu\text{m}$; e, recording microelectrode; c, calomel electrode in sea-water bath; p, neutralized capacity unity-gain electrometer; f, high-cut filter. a, d.c. amplifier used for potential recordings. b, a.c. differential amplifier used for recording bridge balance. A 2000 Hz sine wave was applied to the bridge. From Sibaoka and Eckert [32].

pressure was needed to keep the membrane tight to the opening of a thinner suction pipette, therefore, the Cl^- channels in the membrane inside the pipette could have been injured by excessive stretching of the membrane. As a matter of fact, we failed to record the outward current, when we used a thinner suction pipette like theirs. In conclusion, the hyperpolarization-sensitive Cl^- channels responsible for the negative spike are present anywhere on the cell surface, but most densely clustered around the cytostome.

Oami and Naitoh [33] measured regional membrane impedance changes and localized inward membrane currents associated with the positive spike under Ca^{2+} deficient condition. They found that both impedance decrease and inward current were detected only when the suction pipette was

placed around the cytostome and the tentacle. Furthermore, the positive spike-associated localized membrane currents were always outward through the rest of the membrane. The outward current is a return current of the active inward current through the cytostome region. These results clearly indicate that the depolarization-sensitive Na^+ channels responsible for the positive spike are present restrictedly at the region around the cytostome.

Conduction of the negative spike over the cell surface

Presence of the hyperpolarization-sensitive Cl^- channels all over the cell surface implies a propagation of the negative spike over the cell surface. As a matter of fact, the localized current was

always diphasic. It was first positive then became negative when the suction electrode was around the cytotome, but was first negative then positive when the electrode was on the membrane distant from the cytotome [33]. Since the positive current is an active Cl^- current and the negative current is its passive return current, the positive-negative current sequence at the cytotome region corresponds to the generation of the negative spike and its subsequent propagation away to the aboral region. The negative-positive current sequence at the area distant from the cytotome corresponds to the approaching of the negative spike from the cytotome region and the subsequent generation of the spike in the area.

Voltage-sensitivity of the ionic channels

The outer membrane of *Noctiluca* produces the positive spike in response to an outward current when its membrane potential level has been more or less hyperpolarized, while it produces the negative spike in response to an inward current when the membrane potential level has been more or less depolarized [29]. Conventional two-microelectrode voltage clamp of *Noctiluca* [34] revealed that a step depolarization of the membrane from a holding potential of -80 mV produced a transient inward Na^+ current, which corresponds to the positive spike. The I-V relationship of the current was the so-called N-shape, which is common to other conventional excitable membranes with voltage-sensitive channels. Threshold voltage was about -50 mV and the reversal potential +25 mV. Inactivation of the current was depolarization-dependent. The steady state inactivation curve for the current was the typical S-shape. When the step depolarization was beyond 0 mV, a delayed outward current came to follow the Na^+ inward current. The ion species responsible for the outward current remain unidentified.

On the other hand, a step hyperpolarization of the outer membrane from a holding potential of -20 mV, produced a transient outward Cl^- current, which corresponds to the negative spike. The I-V relationship of the current showed a shape similar to that of the Na^+ inward current but rotated by 180 degrees. The threshold voltage was about -30 mV, and the reversal potential about -

90 mV. The current exhibited hyperpolarization-dependent steady state inactivation.

Modulation of the Na^+ channels by Ca^{2+}

As already mentioned, the positive spike becomes conspicuous when the external Ca^{2+} concentration is lowered. Under voltage clamp condition, the Na^+ inward current became larger and longer as the external Ca^{2+} concentration was lowered, while it became smaller and shorter as the Ca^{2+} concentration was raised. The increasing phase of the inward current was little affected, whereas its decreasing phase was prolonged by lowering the Ca^{2+} concentration. The threshold and reversal potential for the inward current were remained unchanged despite changes in the external Ca^{2+} concentration [30]. These data strongly suggest that external Ca^{2+} modifies the inactivation process of the voltage sensitive Na^+ channels, but not the activation process. Under high Ca^{2+} conditions, inactivation of the Na^+ channels is so fast and the Na^+ conductance increase so small that the positive spike becomes obscure. On the other hand, in low Ca^{2+} , the Na^+ channels are inactivated slowly, so the Na^+ conductance is allowed to become high enough to generate a conspicuous positive spike.

Factors affecting the contractile mechanism of the tentacle

External Ca^{2+} is required for the control of the tentacle movement by the TRPs [28, 29]. Ca^{2+} , therefore, seems to be involved in the activation of the contractile mechanism responsible for the tentacle movement. In order to investigate the intracellular chemical factors activating the contractile mechanism, Oami and Naitoh [12] examined the effects of various chemicals on the detergent-extracted, membrane-disrupted tentacle of *Noctiluca*. The extracted tentacle flexed when the pH of the reactivation medium was lowered (Fig. 11). Maximum flexion was seen at pH 4.0. The extracted tentacle sometimes flexed and coiled if the pH was lowered to below 5.5. Ca^{2+} was much less effective than H^+ in producing the flexion of the extracted tentacle. K^+ , Na^+ and Mg^{2+} were all ineffective in producing the flexion. Addition of ATP with Mg^{2+} or Ca^{2+} had no effect

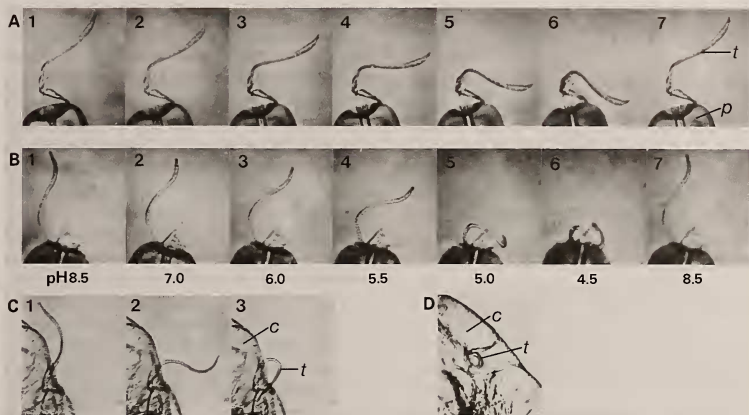


FIG. 11. A and B: Two typical examples of motile responses to H^+ ions in Triton X-100-extracted food-gathering tentacles of the marine dinoflagellate *Noctiluca*. Photographs are magnified images of an extracted tentacle videorecorded 10–20 sec after its subjection to various solutions with different pH values. An extracted tentacle (t) with its basal appendages was held at the tip of a suction pipette (p). After the pH of the reactivation medium had been lowered, the tentacle flexed in A (1–6), whereas in B it flexed (1–4) and finally coiled (5, 6). The tentacle resumed its original shape when the pH was raised again (compare 1 with 7 in both A and B). C: Photographs of a spontaneously moving tentacle of a live specimen of *Noctiluca* in artificial sea water (ASW). 1, extended; 2, half flexed; 3, completely flexed. c, cell body. D: A photograph of a coiled tentacle of a live specimen in Ca^{2+} -rich ASW. From Oami and Naitoh [12].

on the pH-dependent flexion. These results clearly indicate that the contractile mechanism in the tentacle is regulated and energized by H^+ .

The contractile mechanism of the tentacle resembles that of the contractile protein responsible for the contraction of the stalk in *Vorticella*, spasmin, in its ATP independence [35, 36]. However, spasmin is activated by Ca^{2+} as are many other contractile mechanisms. The H^+ -dependent contractile mechanism in the tentacle of *Noctiluca* is very unique among eucaryotic cells. The relationship between the fine structure and the flexion of the tentacle remains to be investigated.

The TRP-tentacle flexion coupling: A hypothesis

As already mentioned, the vacuolar pH is low enough (pH 3.5 [20]) to supply H^+ ions into cytoplasm for activation of H^+ -dependent luminescent mechanism [8]. Similarly, H^+ ions

must be supplied from the vacuole for activation of the H^+ -dependent flexion mechanism of the tentacle. Ca^{2+} requirement for the flexion of the tentacle implies involvement of Ca^{2+} in the release of H^+ from the vacuole into cytoplasm of the tentacle. Considering these facts together with electropysiological evidence, Oami and Naitoh [12] proposed a hypothesis for the coupling mechanism between the TRPs and tentacle movements (Fig. 12). Depolarization-sensitive Ca channels are activated by a slow depolarization in the TRP (pre-spike positive wave) and causes Ca^{2+} influx into the cytoplasm of the tentacle. This increase in the cytoplasmic Ca^{2+} concentration activates a proton transport system in the inner membrane to convey H^+ ions from the vacuole into the cytoplasm. The lowered pH activates the H^+ -dependent contractile mechanism in the tentacle. Activation of the mechanism results in the

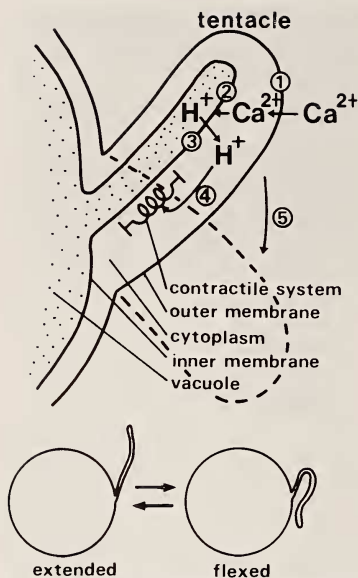


FIG. 12. Schematic representation of the proposed mechanism for bioelectrical control of movement of the food-gathering tentacle in a marine dinoflagellate *Noctiluca miliaris*. External Ca^{2+} is driven into the cytoplasm of the tentacle by a hyperpolarizing spike of the tentacle-regulating potentials [28, 29] through Ca^{2+} channels activated by a slow depolarization preceding the spike (1). The resultant increase in cytoplasmic Ca^{2+} concentration activates a H^+ transport system in the inner membrane facing the flotation vacuole (2). The transport system conveys H^+ from vacuole to the cytoplasm (3). The resultant increase in cytoplasmic H^+ concentration activates the H^+ -sensitive contractile system in the tentacle (4). The activation of the system results in flexion of the tentacle (5). The Ca^{2+} influx ceases when the hyperpolarizing spike terminates. Cytoplasmic Ca^{2+} concentration is decreased by pumping out or sequestering of Ca^{2+} . Thus the H^+ transport system is deactivated. Cytoplasmic H^+ concentration is decreased by pumping out of H^+ or by some other means (not yet identified). The contractile system is then deactivated. The tentacle extends owing to its elasticity. The lower insets show the positions of extended and flexed tentacles relative to the cell body. From Oami and Naitoh [12].

flexion of the tentacle. The tentacle, therefore, continues to flex during the slow depolarization. The rate of the Ca^{2+} influx is suddenly increased by the negative spike due to the increased driving force on Ca^{2+} ions. This causes a rapid increase in the cytoplasmic Ca^{2+} concentration, and in the H^+ concentration thereby. Thus an enhancement of the flexion takes place in association with the negative spike. Sustained hyperpolarization seen after the negative spike (post-spike negative level) causes deactivation of the Ca channels. The Ca^{2+} influx, therefore, ceases. Cytoplasmic Ca^{2+} ions are pumped out or sequestered to lower the cytoplasmic Ca^{2+} concentration back to its normal level. The H^+ transport system is thus deactivated. The cytoplasmic H^+ concentration is then lowered by the pumping out of H^+ ions or by some other means. The contractile system is then deactivated. The tentacle's extension then occurs due to its own elasticity.

BIOELECTRIC CONTROL OF FOOD INTAKE BEHAVIOR

Feeding behavior of *Noctiluca* and its correlation with the TRPs

Motile activity exhibited by *Noctiluca* during its feeding on green algae can be divided into two successive phases, 1) the food gathering phase, in which flexion and extension of the tentacle is repeated for trapping the algae, and 2) the food intake phase, which consists of an initial strong flexion of the tentacle so that its tip plus trapped algae touches the cytostome. Phagocytotic ingestion of the algae through the cytostome accompanied by vigorous cytoplasmic streaming towards the cytostome region then takes place.

During food-gathering, *Noctiluca* exhibits TRPs as described in previous sections. The potential pattern of the TRPs suddenly changes when *Noctiluca* initiates the food intake phase [10].

Experimental induction of the food intake behavior

Nawata and Sibauka [9] looked for various chemical factors in the external solution which could induce food intake behavior in *Noctiluca*. Reduction of SO_4^{2-} concentration to less than 2

mM from its original 30 mM was most effective in inducing motile activity comparable with the food intake behavior. That is, *Noctiluca* showed cytoplasmic aggregation around the cytostome and subsequent formation of empty food vacuoles. A crude extract of the food algae was only 15% effective compared to the reduction of SO_4^{2-} . Reduced glutathione was as effective as the food extract. Some aminoacids, such as valine, methionine, triptophan, leucine, histidine and proline, induced the behavior in 40–0% of the cells examined. External Ca^{2+} , Mg^{2+} , and Cl^- ions were necessary for the induction of the food intake behavior.

The food intake behavior was also induced by pressing the tip of a glass capillary against the cell surface around the cytostome. The capillary was then engulfed by the cell (Fig. 13). Interestingly pressing the pipette against the cell surface other than the cytostome region was not effective in inducing the behavior.

Injection of an inward current into the vacuole also induced the food intake activity similar to lowering SO_4^{2-} concentration.

The food intake behavior and its electrophysiological correlates

Deprivation of SO_4^{2-} from the external solution caused a marked increase in the repetitive frequency of the negative spike and prolongation of the spike, and thereby a final sustained hyperpolarization [10]. The food intake behavior took place when the hyperpolarization exceeded -80 mV and lasted for more than 1 min. The reduced SO_4^{2-} -induced hyperpolarization shifted toward depolarizing direction if the external Cl^- concentration was lowered. This suggests Cl^- -dependence of the sustained hyperpolarization like the negative spike of TRPs.

A membrane hyperpolarization produced by an inward current injection induced the food intake behavior in normal ASW as well as in an ASW with lowered Cl^- concentration. Therefore, a membrane hyperpolarization is a primary cause for induction of the food intake behavior. However, a membrane hyperpolarization did not induce the food intake behavior when the external Ca^{2+} concentration was as low as 10^{-8} M. Na^+ , K^+ and

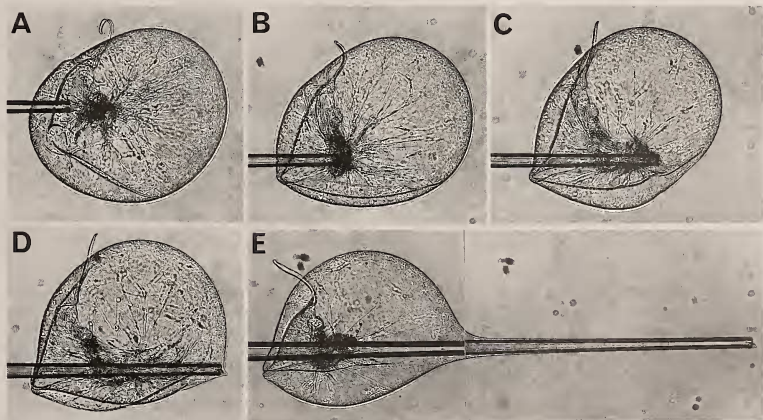


FIG. 13. A series of photomicrographs of a hungry *Noctiluca* swallowing a glass capillary. B, C, D, and E were taken at 5, 14, 17, and 24 min after taking A, respectively. A, tip of the capillary is pressed against the cytostome; B, swallowing of the capillary is initiated; C-D, swallowing is progressing; E, swallowing is at its maximum as the extended cell membrane pushes the capillary back toward the cytostome. Magnification in E is 85% of the others. From Nawata and Sibaoka [9].

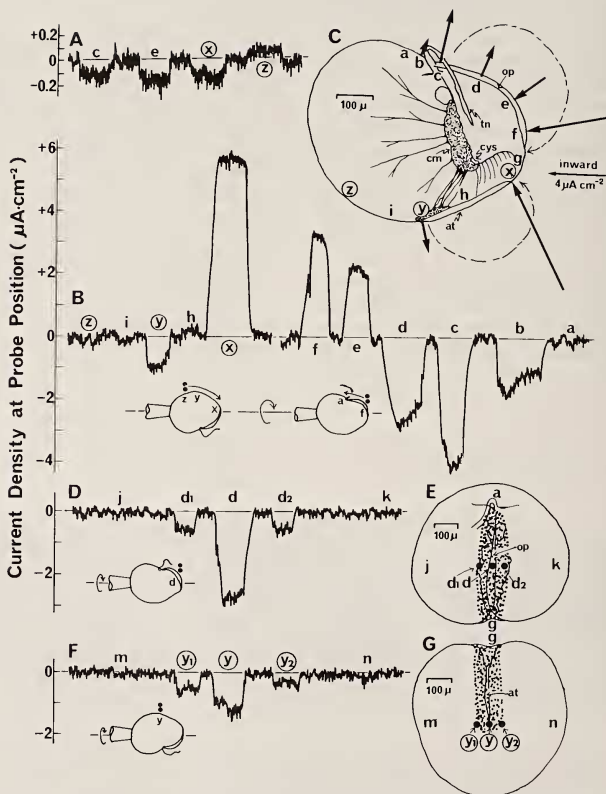


FIG. 14. Localized membrane currents associated with the feeding behavior in *Noctiluca* measured with a vibration current probe. Locations of the probe was indicated by small letters in the schematic drawings of the cell (C, E and G). Trace A: Current recorded before the food intake behavior is experimentally induced. Traces B, D, E and F: Currents recorded 5 to 15 min after the food intake behavior-inducing solution was administered to the cell. B, current recorded along the sulcus; C, distribution pattern of currents along the sulcus depicted on the basis of B. Arrows toward the cell indicate inward currents and those away from the cell outward currents. The length of each arrow is proportional to the current density estimated at each position; D, current recorded across the oral pouch; E, current flowing region (dotted area) estimated from D and some other similar recordings; F, current recorded across the distal end of the apical trough. G, current flowing region (dotted area) estimated from F and others. Nawata and Sibaoka [11].

H⁺ concentration changes had no effect on the food intake behavior.

By employing a vibrating current probe, Nawata and Sibaoka [11] examined localized transmembrane ionic currents on different areas of the cell surface in relation to the feeding behavior. The current entered into the cell through the cytostome region, and it left the cell through both ends of the sulcus (Fig. 14). Current intensity was 7.5 $\mu\text{A}/\text{cm}^2$ at the proximal end of the apical trough during the sustained hyperpolarization. The current intensity decreased if Ca²⁺ and Na⁺ concentrations in the external solution were decreased. Therefore, the current is assumed to be carried by both Na⁺ and Ca²⁺.

Using data collected from the introduction of ⁴⁵Ca to *Noctiluca* during feeding behavior, Nawata and Sibaoka [11] calculated that Ca²⁺ concentration in the cytoplasmic compartment near the cytostome had risen to several μM during hyperpolarization over 1 min. Thus presumably, external Ca²⁺ is driven into the cytoplasm during the hyperpolarization, and regulates the cytoplasmic streaming essential for food ingestion. A role of Ca²⁺ in regulation of protoplasmic streaming and amoeboid movement has long been suggested by many authors [37-40].

DISCUSSION AND CONCLUSION

Control of different effectors, by separate mechanism

In *Noctiluca* the bioluminescent flash is controlled by the FTP generated across the inner membrane, whereas the tentacle movement is controlled by the TRPs generated across the outer membrane. The possession of two control methods for two different effector activities could minimize possible interference between the activities within the single cell. The control of different effector activities by separate mechanisms is also seen in some protozoans. For example, the carnivorous ciliate *Didinium* exhibits two kinds of effector activity, i.e. a ciliary motile response to a mechanical stimulus and extrusion of the extruding organelles in response to its food (mostly *Paramecium*) [41]. The ciliary response is controlled by

Ca²⁺-mediated electrogenesis across the surface membrane like *Paramecium* [1]. Whereas, the extrusion is controlled by a chemosensory mechanism in the tip of the proboscis independently of the electrogenesis across the surface membrane. *Vorticella* shows a ciliary motile response and a contraction of the cell in response to a mechanical stimulus. The ciliary response is controlled by the membrane electrogenesis like other ciliates, whereas the cellular contraction is independent of the membrane electrogenesis [42, 43].

Control of one effector by two separate mechanisms

Both the flash and the contractile mechanisms of *Noctiluca* are activated by H⁺ ions, which are supplied from the intracellular vacuole. It is interesting to note that the flash is always accompanied by an instantaneous coiling of the tentacle. The coiling is regarded as a strong flexion. The tightly coiled tentacle is useless for food-gathering. However, the coiled tentacle is certainly secure from damage caused by mechanical agitation, which triggers the FTP in nature. The tentacle plays a major role in the feeding behavior of *Noctiluca*. The H⁺-dependent contractile mechanism of the tentacle is precisely controlled by the TRPs through the mediation of Ca²⁺ when it exhibits feeding activity. However, when *Noctiluca* faces an emergency (eg. jostling by a big surf), H⁺ ions carried by the FTP directly activate the H⁺-dependent contractile mechanism to produce coiling of the tentacle.

Biological significances in the distribution of the ionic channels within a single membrane

In *Noctiluca* H⁺ channels responsible for the FTP are distributed all over the inner membrane. This distribution contributes to the propagation of the flash along the inner membrane from the stimulus site. The Cl⁻ channels responsible for the negative spike are distributed all over the outer membrane, but the channel density is higher in the area around the tentacle than the rest of the membrane area. The denser distribution of the Cl⁻ channels brings about a higher rate of Ca²⁺ influx into the tentacle for its rapid flexion. Overall distribution of the Cl⁻ channels in the outer membrane somehow relates to the generation of

the sustained hyperpolarization, which initiates the food intake behavior. However, the biological significance of the localized distribution of the Na⁺ channels, responsible for the positive spike around the tentacle region, remains to be investigated. The distribution of the ionic channels in a single membrane plays an important role in the precise control of the effector activity. This has been well documented in *Paramecium* and some other ciliate protozoans [1].

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